Calmodulin content does not change following hormone-induced meiosis reinitiation in starfish oocytes

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Summary. Activator-deficient phosphodiesterase from bovine heart was stimulated by heat-treated dialyzed extracts from starfish oocytes. The same dose-response curve was obtained with extracts from oocytes which had or had not been released from prophase block by 1-methyladenine. Such extracts were shown to contain calmodulin by affinity chromatography on troponin I covalently bound to sepharose and SDS polyacrylamide gel electrophoresis. The results suggest that no change in calmodulin content occurs in starfish oocyte following meiosis reinitiation.

In starfish, full grown oocytes are arrested at the late prophase of the 1st meiotic division. Meiosis is reinitiated by a relay hormone produced by the follicle cells under the influence of a hormonal peptide[1,2]. The relay hormone has been identified as 1-methyladenine[3]. It has been shown that 1-methyladenine acts on stereospecific receptors localized on the oocyte membrane to trigger within seconds a transient increase of free Ca^{++} concentration within the oocyte[4-6]. On the other hand increase of protein kinase activity is already noticeable 2 min after starting the hormonal treatment[7]. In mammals a calcium-binding protein referred to as calmodulin has been shown to exhibit multiple calcium-dependent regulatory activities, including activation of brain adenylate cyclase[8] and protein kinase[9]. Recently calmodulin was purified to homogeneity from sea urchin oocytes according to a procedure including affinity chromatography on troponin I covalently bound to sepharose[10]. These findings prompted us to investigate possible changes in calmodulin activity which might occur in starfish oocytes as a consequence of 1-methyladenine induced release of prophase block.

Material and methods. Activator-deficient cyclic nucleotide phosphodiesterase was prepared from bovine heart according to Teo and Wang[11]. Troponin I was prepared from rabbit skeletal muscle according to Head et al.[12] and coupled to activated sepharose 4 B according to Axen et al.[13]. *Asterias rubens* oocytes were prepared free of follicle cells as described previously[5], washed rapidly with 20 mM Tris-HCl, 1 mM $MgCl_2$, 1 mM imidazole, pH 7.5 (buffer A), resuspended in the same buffer and submitted to 20 strokes of a hand homogeniser fitted with a teflon pestle. After 10 min centrifugation at 20,000 × g the supernatant was collected and allowed to stand for 6 min at 96 °C (a treatment which abolishes endogenous phosphodiesterase activity): precipitated proteins were removed by centrifugation and the supernatant dialysed at 4 °C for 24 h and subsequently spun at 50,000 × g for 30 min. The clarified supernatant was then processed according to the 2-step procedure described by Head et al. for the purification of sea urchin occyte calmodulin[10] (briefly: ion exchange followed by affinity column chromatography on troponin I covalently bound to sepharose). SDS polyacrylamide gel electrophoresis modified for gel slabs was performed according to Laemmli[14]. The activator protein was assayed on the basis of its ability to stimulate a fixed amount of the activator-deficient bovine heart phosphodiesterase. The enzyme activity was measured by the radiochemical, 2-step assay, according to Thomson and Appleman[15]. Standard reaction mixtures contained 100 µM $CaCl_2$ 10 µM cAMP, 10 mM tris-HCl pH 7.5, 0.5 mM $MgCl_2$, 0.5 mM imidazole and 10 µM ^{3}H-cAMP (20,000 cpm) in 300 µl. Brain calmodulin was a generous gift of Dr Cheung, Memphis, USA.

Results and discussion. Figure 1 shows that dialyzed extracts of starfish oocytes are able to stimulate the activity of activator-deficient phosphodiesterase from bovine heart. As in the case of purified bovine hart protein-activator, the activation of the enzyme produced by dialyzed oocytes extracts can be suppressed by addition of EGTA[11]. Subsequent addition of Ca^{++} in excess of EGTA restores the initial rate of enzymic reaction. To demonstrate the presence of authentic calmodulin in the dialyzed extracts these were fractionated according to Head et al.[10]. A fraction

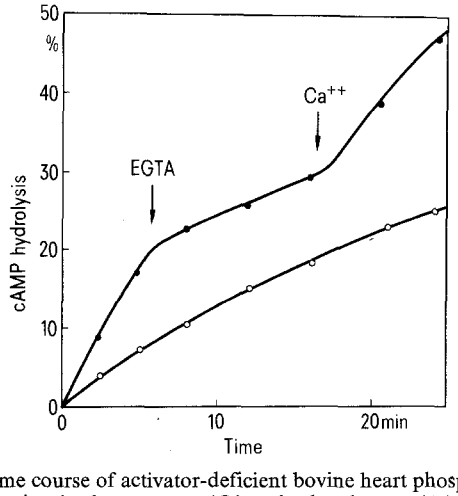

Fig. 1. Time course of activator-deficient bovine heart phosphodiesterase reaction in the presence (●) or in the absence (○) of heat-treated dialyzed extract from starfish oocytes. 100 µM Ca^{++} was present initially in both incubation mixtures. The incubation mixture containing the starfish extract was ajusted to 0.4 mM EGTA at 6 min and to 1 mM Ca^{++} at 17 min. Aliquots were removed at indicated times and analyzed for cAMP hydrolysis.

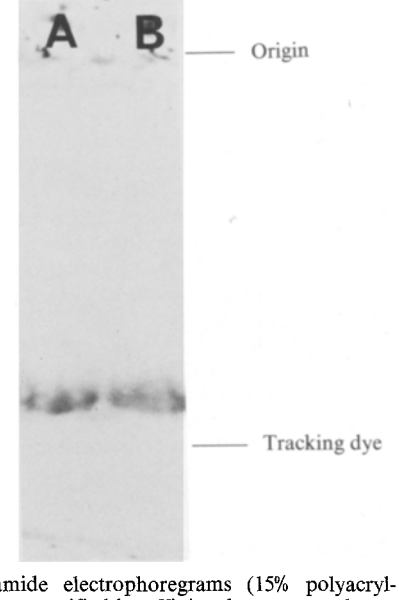

Fig. 2. SDS polyacrylamide electrophoregrams (15% polyacrylamide) of starfish activator purified by affinity chromatography on troponin I covalently bound to sepharose (A) and of authentic brain calmodulin (B). Gels were stained in Coomassie Brilliant Blue R 250.

eluting from the DE 52 column at 0.3 M NaCl was found to contain a factor which alike calmodulin was retained in the presence of Ca^{++} on troponin I covalently bound to sepharose and which was specifically eluted in the presence of an excess of EGTA[10]. As shown on figure 2 this factor co-migrates with authentic brain calmodulin in SDS polyacrylamide gel electrophoresis.

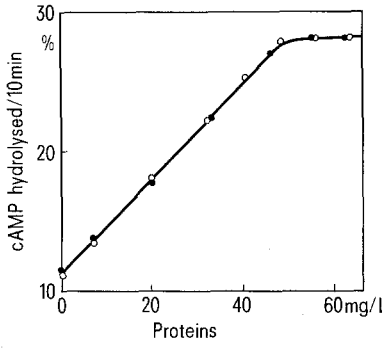

Fig. 3. Dose-response curve for the activation of activator-deficient bovine heart phosphodiesterase by extracts of *Asterias rubens* oocytes. 2 identical oocyte suspensions were used. One received $2 \cdot 10^{-6}$M 1-methyladenine for 15 min. The 2nd served as control. Heat-treated dialyzed extracts were prepared under the same conditions as described in a material and methods, and adjusted to various protein concentrations by dilution with buffer A. The enzyme was assayed with various amounts of the dialyzed heat treated extracts from controls (○) or hormone-treated oocytes (●).

Figure 3 shows that the activation increases rather linearly with the amount of starfish extract to a maximal value. When increasing amounts of starfish calmodulin purified by affinity chromatography were substituted for the dialyzed extracts the same maximal stimulation of the activator-deficient beef heart phosphodiesterase was obtained, which suggested that calmodulin was the only activator of the mammalian enzyme present in the dialyzed starfish extracts. As already reported for the sea urchin oocyte, no sensitivity of starfish oocyte cyclic nucleotide phosphodiesterase towards Ca^{++} could be demonstrated (data not shown). Moreover no change in phosphodiesterase activity occured within oocytes following 1-methyladenine treatment.

To investigate possible changes in calmodulin content or activity following meiosis reinitiation, heat-treated dialyzed extracts were made under strictly identical conditions from starfish oocytes which had or had not been treated with 1-methyladenine for 15 min, a period which is long enough to elicit the appearance of 'maturation-promoting factor' (MPF) within starfish oocyte cytoplasm. As shown in figure 3 doses and responses were found to be strictly superimposable in both cases. This result shows that MPF activity and release from prophase block cannot be correlated with any change in calmodulin content or intrinsic activity (at least towards mammalian phosphodiesterase). It remains possible, however, that there are changes in calmodulin localization within the oocyte like those which have been described in mammalian cells during the time course of mitosis[17], or in the Ca^{++}-calmodulin complex level. These are under investigation.

1 H. Kanatani, Science *146*, 1177 (1964).
2 A.W. Schuetz and J.D. Biggers, Exp. Cell Res. *46*, 642 (1967).
3 H. Kanatani, H. Shirai, K. Nakanishi and T. Kurokawa, Nature *221*, 273 (1969).
4 M. Dorée, P. Guerrier and N.J. Léonard, Proc. natl Acad. Sci. USA *73*, 1669 (1976).
5 M. Dorée and P. Guerrier, Exp. Cell Res. *91*, 341 (1975).
6 M. Dorée, M. Moreau and P. Guerrier, Exp. Cell Res. *115*, 251 (1978).
7 P. Guerrier, M. Moreau and M. Dorée, Molec. cell. Endocr. *7*, 137 (1977).
8 C.O. Brostrom, Y.C. Huang. M.Mc. Breckerridge and D.J. Wolff, Proc. natl Acad. Sci. USA *72*, 64 (1975).
9 R. Dabrowska, J.M.F. Sherry, D.K. Aromatoris and D.J. Hartshorne, Biochemistry *17*, 253 (1978).
10 J.F. Head, S. Mader and B. Kaminer, J. Cell Biol. *80*, 211 (1979).
11 T.S. Téo and J.H. Wang, J. biol. Chem. *248*, 5950 (1973).
12 J.F. Head, R.A. Weeks and S.V. Perry, Biochem. J. *161*, 465 (1977).
13 R. Axen, J. Porath and S. Ernback, Nature *214*, 1302 (1967).
14 U.K. Laemmli, Nature *227*, 680 (1970).
15 W.J. Thompson and M.M. Appleman, Biochemistry *10*, 311 (1971).
16 T. Kishimoto and H. Kanatani, Nature *260*, 321 (1976).
17 M.J. Welsh, J.R. Dedman, B.R. Brinkley and A.R. Means, Proc. natl Acad. Sci. USA *75*, 1867 (1978).

Interruption of acoustic communication and mating in a leafhopper and a planthopper by aerial sound vibrations picked up by plants

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Summary. Each sex of the cotton leafhopper and the rice brown planthopper communicates with the opposite sex by certain specific sound vibrations which travel through the plant surface and lead to mating. External sounds of certain frequencies, viz., 200 c/sec, generated by a harmonium or an audio-oscillator are picked up from the air by the plants and interrupt the acoustic communication as well as mating of the insects. Notes with harmonics are effective whereas pure notes are ineffective.

Various auchenorrhynchous homopterous insects have been reported to emit acoustic signals[2-8] which, in certain species, travel through the host plant surface and mediate the orientation as well as the approach of the males to the females for mating. The possibility of interrupting the acoustic communication and mating of these insects by external sounds has been investigated in our work on the leafhopper *Amrasca devastans* (Dist), a cotton pest, and the brown planthopper *Nilaparvata lugens* Stål, a rice pest.

The patterns of acoustic signals from the males and females of the brown planthopper have been studied by Ichikawa[8]. The sounds emitted by the cotton leafhopper were recorded